Monaldi Arch Chest Dis
1998; 53: 3, 268–273

ORIGINAL ARTICLE

Cigarette smoking does not increase hydrogen peroxide levels in expired breath condensate of patients with stable COPD

D. Nowak, M. Kasielski, T. Pietras, P. Białasiewicz, A. Antczak

ABSTRACT: *Cigarette smoking does not increase hydrogen peroxide levels in expired breath condensate of patients with stable COPD. D. Nowak, M. Kasielski, T. Pietras, P. Białasiewicz, A. Antczak.*

Cigarette smoking is the most common factor responsible for the development of chronic obstructive pulmonary disease (COPD) leading to oxidant overload in the lower airways because of the presence of oxidants in cigarette smoke and recruitment and activation of pulmonary phagocytes. In this study we intended to determine whether: 1) patients with stable COPD have higher H_2O_2 levels in expired breath condensate than healthy nonsmoking subjects and 2) whether cigarette smoking increases H_2O_2 exhalation in patients with stable COPD. The H_2O_2 content of the expired breath condensate of 17 healthy nonsmoking subjects and 38 patients (10 current smokers, 17 exsmokers and 11 who have never smoked) with stable COPD (forced expiratory volume in one second (FEV1) 63.3±15.5% of predicted value) was measured spectrofluorimetrically (homovanillic acid method).

The mean H_2O_2 concentration in the expired breath condensate of COPD subjects was 10-times higher than that found in healthy controls (0.55±0.69 μM *versus* 0.05±0.07 μM, $p<0.005$). There were no significant differences between H_2O_2 levels found in current smokers with COPD (0.44±0.56 μM) and COPD subjects who have never smoked (0.49±0.70 μM). No correlation was found between expired H_2O_2 and daily cigarette consumption or cumulative cigarette consumption in current smokers or exsmokers with COPD.

These findings demonstrate that subjects with stable chronic obstructive pulmonary disease exhibit increased H_2O_2 generation in the airways and that cigarette smoking does not increase H_2O_2 production.

Monaldi Arch Chest Dis, 1998; 53: 3, 268–273.

Keywords: Breath condensate, chronic obstructive pulmonary disease, cigarette smoking, hydrogen peroxide.

Dept of Pneumology and Allergology, Medical University of Lodz, Lodz, Poland.

Correspondence: D. Nowak, Dept of Pneumology and Allergology, Medical University of Lodz, Kopcinskiego st 22, 90–153 Lodz, Poland.

Received: July 11 1997; accepted after revision January 31 1998

Cigarette smoking is recognized as the most important factor responsible for the occurrence of chronic obstructive pulmonary disease (COPD) [1]. Cigarette smokers have increased numbers of macrophages and neutrophils in their lower airways [2, 3]. These cells release large amounts of reactive oxygen species, including hydrogen peroxide (H_2O_2) [2, 4], that may inactivate α_1-proteinase inhibitor causing proteolytic lung destruction that results in the development of pulmonary emphysema [5, 6].

Cigarette smoke contains many reactive oxygen species and in addition aqueous solutions of cigarette smoke generate H_2O_2 [7, 8]. H_2O_2 can cross cell membranes and could be converted into the highly cytotoxic hydroxyl radical in the presence of iron and superoxide radicals [9]. Some of the H_2O_2, not decomposed by antioxidant enzymes, may evaporate from the alveolar lining fluid and be excreted with the expiratory air.

Patients with COPD and asymptomatic cigarette smokers have been reported to exhale increased amounts of H_2O_2 compared to healthy nonsmokers [10, 11]. Moreover, infectious exacerbation of COPD has been shown to result in a further rise in H_2O_2 levels [11]. Since cigarette smoke causes influx and activation of pulmonary phagocytes, it is possible that COPD patients who still smoke have higher concentrations of H_2O_2 in expired air than exsmokers or those COPD subjects who have never smoked. Recent studies have, however, provided completely different data, showing that COPD patients who still smoke can exhale lower amounts of H_2O_2 than exsmokers with stable COPD [11]. This suggests that tobacco smoke can induce some compensatory mechanisms providing protection against reactive oxygen species including H_2O_2. However, the above mentioned differences in H_2O_2 exhalation between COPD smokers and exsmokers are based on the analysis of 12 patients (4 smokers, 8 exsmokers) so a bias cannot be excluded [11].

In this study, therefore, we wanted to determine the influence of cigarette smoking on H_2O_2 levels in the expired breath condensate of a larger group of patients with stable COPD. We report herein that COPD patients exhale ten-times more H_2O_2 than healthy nonsmoking subjects and that this variable does not correlate with the cigarette smoking status.

Material and methods

Study populations

The study subjects included 17 healthy volunteers and 38 patients with COPD (table 1) who had not suffered from any infectious diseases for at least 3 months prior to the study. The healthy never smoking subjects, who had no history of respiratory or atopic diseases were members of our medical staff and were free of any medication. Routine physical examination showed nothing abnormal.

Forty-two consecutive COPD patients without any concomitant diseases were selected from 125 COPD subjects whose data were found in the Medical University Outpatient Department COPD register. These patients had not taken inhaled or oral corticosteroids within the last three months. Subjects were asked to stop all medication except for short-acting β_2-agonists (salbutamol or fenoterol on demand up to 4 puffs·day^{-1}), anticholinergics (inhaled ipratropium bromide 0.12 mg·day^{-1}) and theophylline (600 mg·day^{-1}), and to come to the clinic after a four week washout period to collect expired breath condensate and perform lung function tests. The intake of any drugs (*e.g.* *N*-acetylcysteine, ambroxol, vitamins) with possible antioxidant activity [12–15] was not allowed during the study.

Spirometry was performed using a Flowscreen (Erich Jaeger GmbH, Würzburg, Germany) equipped with software compatible to the American Thoracic Society standards [16], between 08:00 and 09:00 h. Subjects refrained from using inhaled drugs (β_2-agonists and anticholinergics) and oral theophylline for 6 and 12 h before lung function measurement, respectively. The criteria for inclusion were an ability to stop medication other than the above recommended therapies and an increase in the forced expiratory volume in one second (FEV1) of less than 10% of the predicted value 15 min after taking 200 µg salbutamol (2 puffs from a metered dose inhaler). The predicted values were derived from the European Community for Steel and Coal statement [17]. Thirty eight of the COPD patients fulfilled these criteria. Ten of them were current smokers, 17 were exsmokers and 11 had never smoked. The spirometric data were similar for all COPD subgroups (table 2).

The never smoking COPD patients (3 males, 8 females) were professionally exposed for >20 yrs to factors that are known to predispose to COPD.

Table 1. – Characteristics of the study subjects

	Healthy never smokers	COPD patients
Number	17	38
Age yrs	55±7	60±9
Sex M:F	10:7	20:18
FEV1 % pred	99±4	63.3±15.5**
FEV1/FVC	80.3±1.4	57.6±12.9**
FEV1 reversibility % pred	1.3±1.2	5.0±3.4

Data are expressed as mean±SD. COPD: chronic obstructive pulmonary disease; M: male; F: female; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; **: p<0.01 *versus* appropriate value of normal subjects.

Table 2. – Characteristics of the chronic obstructive pulmonary disease patient subgroups

	Current smokers	Exsmokers*	Never smokers
Number	10	17	11
Age yrs	52±11	65±6	62±9
Sex M:F	6:4	11:6	3:8
Current cigarette consumption cigarettes·day^{-1}	14±7	0	0
Cumulative cigarette consumption pack-yrs	22.4±15.2	27.6±20.4	0
FEV1 % pred	62.4±12.5	64.3±16.0	62.4±18.2
FEV1/FVC	58.2±8.1	57.0±14.9	58.1±14.3
FEV1 reversibility % pred	5.5±3.5	4.3±3.9	5.7±2.5

Data are expressed as mean±SD. *: time since smoking cessation varied from 6–396 months, mean 126±114 months. M: male; F: female; FEV1: forced expiratory volume in one second; FVC: forced vital capacity.

One male was a welder, one a grinder and another worked in a coal merchant's shop. The females were textile industry workers (dye-works, chemical warehouses or spinning rooms).

All patients were asked about their smoking and disease history and their medical documentation was carefully analysed. The number of exacerbations·yr^{-1} in the 2 yrs prior to the visit in the whole COPD group ranged from 1–8 (mean 3±2). In the current smoker, exsmoker and never smoker COPD patients the mean number of exacerbations·yr^{-1} reached 4±2, 3±2, and 4±2, respectively. The duration of COPD (calculated from the date a firm diagnosis was made) ranged from 24–436 months, (mean 125±91) for the whole group, and 76±52, 165±105, and 107±73 months for current smoker, exsmoker and never smoker COPD patients, respectively. This study was approved by the Medical University Ethics Committee and all subjects gave informed consent before participation.

Collection of air condensate

The expired breath condensate was collected just prior to the lung function tests, as described previously [10, 18]. Briefly, patients were asked to breathe out spontaneously through a mouthpiece with a saliva-trap connected to a collecting tube and then to breathe in with the mouthpiece removed for 20 min. The collecting part of the tube (170 cm in length) was covered with ice and salt so that the temperature around the tube ranged from -6–0°C, allowing all H_2O_2 present in the exhaled air to be condensed [19]. Each subject wore a noseclip and rinsed their mouth with distilled water just before and at 7 and 14 min of collection, in order to reduce H_2O_2 evaporation from saliva [19] and nasal spaces [10]. At the end of collection, the tube was removed from the polystyrene foam container and 2–5 mL aliquots of condensate were transferred to Eppendorf tubes and stored at -80°C (for not more than 7 days) until H_2O_2 measurements were made. All collections were performed between 09:00 and 11:00 h

and subjects refrained from cigarette smoking for 12 h before the visit. If the patient failed to refrain from smoking or stop medication (as described above) the breath condensate was not collected and the visit was rescheduled within the next 1–7 days.

Measurement of hydrogen peroxide

The concentration of H_2O_2 in expired breath condensate was measured according to the method of RUCH *et al.* [20]. Briefly, 600 μL expired breath condensate was mixed with 600 μL of horseradish peroxidase solution (1 $U \cdot mL^{-1}$) containing 100 μM homovanillic acid and was incubated for 60 min at 37°C. The sample was then mixed with 150 μL 0.1 M glycine-NaOH buffer (pH 12.0) containing 25 mM ethylenediaminetetraacetic acid. The homovanillic acid oxidation product, used as a measure of the amount of H_2O_2 present, was determined spectrofluorimetrically using a Perkin Elmer Luminescence Spectrometer LS-50B (Norwalk, CT, USA). Excitation was at 312 nm and emission was measured at 420 nm. Readings were converted into concentration (nM) using the regression equation

$$Y=67.64(X-X_0)$$

where Y=nmol $H_2O_2 \cdot L^{-1}$ in the expired breath condensate, X=intensity of emission at 420 nm expressed in arbitrary units, and X_0=intensity of emission given by a reference sample receiving distilled water instead of breath condensate, obtained from three series of experiments with 10 increasing (0.0125–25 μM) H_2O_2 concentrations. The lower limit of H_2O_2 detection was 83 nM and the calibration curve was linear up to a concentration of 16.7 μM H_2O_2. For readings that gave results below the sensitivity of the method, the H_2O_2 concentration in expired breath condensate was assumed to be 0 nM.

Statistical analysis

Data from subjects are expressed as the mean±SD. Data from healthy subjects and COPD patients (including three COPD subgroups) were analysed using a t-test to compare independent samples with separate variance estimation. A p-value<0.05 was considered to be significant. Pearson correlation was used to determine the relationships between the measured variables.

Results

In healthy, never smoking subjects, the mean H_2O_2 concentration in expired breath condensate was 0.05±0.07 μM (n=17). In 11 (65%) healthy controls (7 male, 4 female) there was no H_2O_2 detectable. Patients with stable COPD exhibited a ten-times higher concentration of H_2O_2 ($p<0.005$) than the healthy nonsmokers (fig. 1). The mean H_2O_2 level in the expired breath condensate reached 0.55±0.69 μM. However, 13 (34%) COPD patients (7 male, 6 female) revealed no detectable H_2O_2 levels. Four of

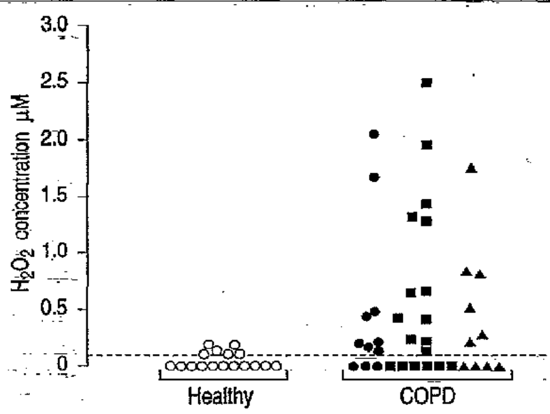


Fig. 1. – Concentration of H_2O_2 in expired breath condensate of healthy never smokers (○) and chronic obstructive pulmonary disease (COPD) subjects (●: never smokers; ▲: current smokers and ■: exsmokers). Individual results below the sensitivity of the H_2O_2 determination (0.083 μM; ----) were assumed to be 0 μM. Mean H_2O_2 levels found in current smoking, exsmoking and never smoking COPD subjects did not differ significantly between each other and were 0.44±0.57, 0.66±0.77 and 0.49±0.70 μM, respectively. However, the mean levels found in healthy controls and the whole COPD group did differ significantly and were 0.05±0.07 μm and 0.55±0.69 μm, respectively, $p<0.005$.

them were current smokers, 6 exsmokers and 3 had never smoked. COPD patients who were permanent smokers revealed a tendency to exhale less H_2O_2 than COPD exsmokers (0.44±0.57 *versus* 0.66±0.77 μM), however this difference was not significant. Patients who had never smoked (n=11) had 0.49±0.70 μM H_2O_2 in their expired breath condensate. Male COPD patients showed similar values to female subjects (0.53±0.65, n=20 *versus* 0.57±0.72 μM, n=18).

A significant correlation was found between H_2O_2 in the expired breath condensate and FEV_1 (% predicted) in COPD exsmokers and those who had never smoked ($r=0.61$, $p<0.01$ and $r=0.61$, $p<0.05$) and disease duration in the exsmoker subgroup ($r=-0.51$, $p<0.05$) (fig. 2.) No correlation was found between the expired H_2O_2 concentration and cumulative cigarette consumption in both smoking and exsmoking COPD subjects ($r=0.15$ and $r=-0.11$). There was no correlation between H_2O_2 levels and present cigarette consumption in COPD patients who still smoked ($r=-0.09$). H_2O_2 also did not correlate with time from cessation of smoking in COPD exsmokers ($r=0.27$). There was no association between the levels of exhaled H_2O_2 and the age of the COPD subjects or healthy controls ($r=0.24$, $r=0.26$). Neither the number of exacerbations per year during the 2 yrs prior to the visit nor the disease duration correlated with the exhaled H_2O_2 in the whole COPD group ($r=-0.08$, $r=-0.23$).

Discussion

In this study, patients with stable COPD had a higher mean H_2O_2 concentration in their expired breath condensate than healthy, never smoking subjects. This is consistent with the recent findings of DEKHUIJZEN *et al.* [11] and provides evidence for oxidant overload in the airways of COPD patients.

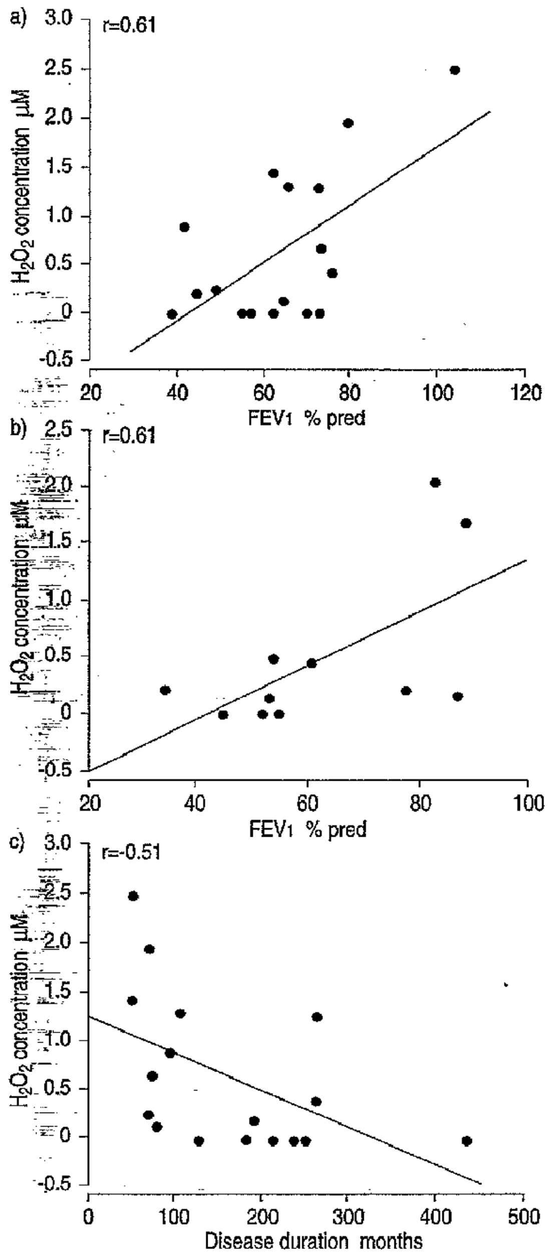


Fig. 2. – The significant correlation between the expired breath H_2O_2 concentration and forced expiratory volume in one second (FEV1) expressed as per cent predicted in a) exsmoking chronic obstructive pulmonary disease (COPD) subjects ($p<0.01$) and b) never smoking COPD subjects ($p<0.05$). There was a significant inverse correlation ($p<0.05$) between the expired H_2O_2 and disease duration in exsmoking COPD subjects (c).

However, there were twice as many negative H_2O_2 readings in our COPD group as in the previous study [11]. This may result from the use of a saliva trap, nose clip and rinsing of the mouth with distilled water just before and during collection of expired breath condensate. In addition, patients involved in the study used inhaled short-acting β_2-agonists, anticholinergics and theophylline. The inhibition of the cellular response to inflammatory mediators by these drugs [21, 22] may also be responsible for some of the negative H_2O_2 results, although one might expect that H_2O_2 exhalation would be higher after discontinuation of the above medication.

Our results support the hypothesis that inflammatory processes within the airways could lead to increased H_2O_2 exhalation. This has been shown for subjects with adult respiratory distress syndrome [19, 23, 24], pneumonia [24], bronchial asthma [18, 25], and asymptomatic cigarette smokers [10]. Various data indicate that inflammatory cells, such as alveolar macrophages and polymorphonuclear leukocytes, seem to be the main source of exhaled H_2O_2. Both asymptomatic cigarette smokers and subjects with COPD have increased numbers of these cells in bronchoalveolar lavage fluid [2, 3, 26]. These cells are activated and subsequently produce more reactive oxygen species, including H_2O_2, than cells obtained from healthy nonsmoking subjects [2, 3]. H_2O_2 can also originate from lung microsomes and mitochondria. The contribution of H_2O_2 from this source may be significant in hyperoxic conditions [27].

Cigarette smoke contains a lot of reactive oxygen species and aqueous solutions of cigarette smoke generate H_2O_2 [7, 8]. However, our COPD smokers refrained from smoking for 12 h before condensate collection. Therefore, it is unlikely that the H_2O_2 measured in breath condensate originated directly from cigarette smoke. There is catalase and glutathione peroxidase activity in the alveolar lining fluid [26, 28] which could decompose H_2O_2 derived from cigarette smoke during the 12 h preceding condensate collection. In addition, exhaled ethane (an alkane byproduct of lipid peroxidation) decreased to baseline levels 3 h after the last cigarette in cigarette smokers, but this value was still higher than that of never smoking subjects [29].

The presence of H_2O_2 in expired breath condensate is a result of several processes including production of H_2O_2, diffusion through cell membranes, decomposition by antioxidant enzymes (catalase, glutathione peroxidase) and evaporation from the alveolar lining fluid. Alveolar macrophage-derived catalase is the main enzyme which decomposes H_2O_2 in the alveolar lining fluid [28]. Cigarette smoke causes influx and increased production of reactive oxygen species by macrophages and polymorphonuclear leukocytes in the lower airways [2, 3]. The increased amounts of exhaled H_2O_2 in asymptomatic cigarette smokers can reflect the pulmonary oxidant overload [10]. However, some components of cigarette smoke, *i.e.* acrolein, are cytotoxic for alveolar macrophages and this may cause the leakage of intracellular enzymes [30, 31]. This may lead to an increase in the catalase activity in the alveolar lining fluid of cigarette smokers [32] and consequently to increased H_2O_2 decomposition. The rise in glutathione level may also be responsible for H_2O_2 decomposition since bronchoalveolar lavage fluid obtained from COPD patients who were current smokers revealed elevated concentrations of reduced and total glutathione [26]. Thus, the pro-oxidant

activity of cigarette smoke and the simultaneous stimulation of the antioxidant defence against H_2O_2 may explain the lack of correlation between the intensity of cigarette smoking and H_2O_2 levels in the breath condensates of current smokers and exsmokers with COPD. This is consistent with studies showing no correlation between exhaled ethane and cigarette consumption nor between breath condensate H_2O_2 levels and smoking status, and urinary cotinine levels in cigarette smokers [10, 15].

Exposure of lung tissue to various inflammatory mediators, including reactive oxygen species, may cause a secondary rise in antioxidant defence [33–35] with subsequent H_2O_2 decomposition. It is possible that patients with chronic airway inflammation may exhale lower amounts of H_2O_2 than those with acute inflammatory response in the lung. Our observation that H_2O_2 levels in breath condensate correlated negatively with disease duration in the COPD exsmokers subgroup confirms this hypothesis to some extent. However, COPD subjects with a higher FEV1 may represent those with lower inflammatory damage to the lungs and a lower secondary rise in the antioxidant enzymes activity. This may explain the positive correlation between H_2O_2 levels and the FEV1 of exsmokers and never smoking subjects. Patients with exacerbation of COPD exhale more H_2O_2 than those with stable disease [11]. Patients included in this study had not suffered from any COPD exacerbation for the last three months prior to recruitment. Since the number of exacerbations per year did not correlate with H_2O_2 in the breath condensates one may suppose that H_2O_2 exhalation decreases to the characteristic COPD stable phase level during the 3 month period after exacerbation.

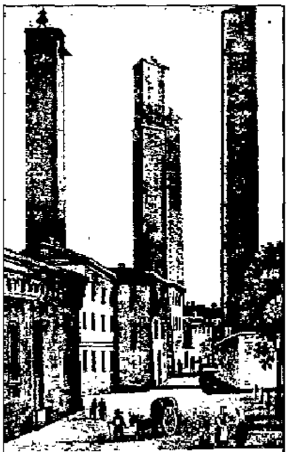
In conclusion, we found that patients with stable chronic obstructive pulmonary disease had elevated H_2O_2 concentrations in their expired breath condensate. H_2O_2 levels did not correlate with cigarette smoking status. Moreover, current smokers with chronic obstructive pulmonary disease did not exhale more of H_2O_2 than nonsmoking chronic obstructive pulmonary disease subjects. Thus, measurement of H_2O_2 could be a simple noninvasive method to demonstrate enhanced generation of reactive oxygen species in the airways of chronic obstructive pulmonary disease patients.

Acknowledgements: The authors wish to acknowledge L. Carati (Zambon Group, Milano, Italy) for his support.

References

1. Sherrill DI, Lebowitz MD, Burrows B. – Epidemiology of chronic obstructive pulmonary disease. *Clin Chest Med* 1990; 11: 375–388.
2. Baughman RP, Corser BC, Strohofer S, Hendricks D. – Spontaneous hydrogen peroxide release from alveolar macrophages of some cigarette smokers. *J Lab Clin Med* 1986; 107: 233–237.
3. Hunninghake GW, Crystal RG. – Cigarette smoking and lung destruction: accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983; 128: 833–836.
4. Ludwig PW, Hoidal JR. – Alterations in leukocyte oxidative metabolism in cigarette smokers. *Am Rev Respir Dis* 1982; 126: 977–980.
5. Stockley RA. – Alpha1-antitrypsin and the pathogenesis of emphysema. *Lung* 1987; 165: 61–77.
6. Nowak D. – The comparative study of reactive oxygen species generated by polymorphonuclear leukocytes as α_1-proteinase inhibitor inactivators - possible application for antioxidant prevention of emphysema. *Arch Immunol Ther Exp* 1988; 36: 71–79.
7. Nakayama T, Church DF, Pryor WA. – Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. *Free Radical Biol Med* 1989; 7: 9–15.
8. Pryor WA, Prier DG, Church DF. – Electron-spin resonance study of mainstream and side stream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ Health Perspect* 1985; 47: 345–355.
9. Pryor WA. – Oxyradicals and related species: their formation, lifetimes and reactions. *Ann Rev Physiol* 1986; 48: 657–667.
10. Nowak D, Antczak A, Król M, *et al.* – Increased content of hydrogen peroxide in expired breath of cigarette smokers. *Eur Respir J* 1996; 9: 652–657.
11. Dekhuijzen PNR, Aben KKH, Dekker I, *et al.* – Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996; 154: 813–816.
12. Moldeus P, Cotgreave IA, Berggren M. – Lung protection by a thiol-containing antioxidant: *N*-acetylcysteine. *Respiration* 1986; 50 (Suppl. 1): 31–42.
13. Nowak D, Antczak A, Kr. Berggren M. – Antioxidant properties of Ambroxol. *Free Radical Biol Med* 1994; 16: 517–522.
14. Felix K, Pairet M, Zimmermann R. – The antioxidative activity of the mucoregulatory agents: ambroxol, bromhexine and *N*-acetyl-L-cysteine. A pulse radiolysis study. *Life Sci* 1996; 59: 1141–1147.
15. Do BKQ, Garewal HS, Clements NC, Peng YM, Habib MP. Exhaled ethane and antioxidant vitamin supplements in active smokers. *Chest* 1996; 110: 159–164.
16. American Thoracic Society. – Standardization of spirometry 1987 update. *Am Rev Respir Dis* 1987; 136: 1285–1296.
17. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. – Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests. European Community for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993; 6 (Suppl. 16): 5–40.
18. Antczak A, Nowak D, Shariati B, Król M, Kurmanowska Z. Increased hydrogen peroxide and TBA-reactive products in expired breath condensate of asthmatic patients. *Eur Respir J* 1997; 10: 1235–1241.
19. Sznajder JI, Fraiman A, Hall JB, *et al.* – Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. *Chest* 1989; 96: 606–612.
20. Ruch W, Cooper PH, Baggiolinii M. – Assay of H_2O_2 production by macrophages and neutrophils with homovanillic acid and horseradish peroxidase. *J Immunol Meth* 1983; 63: 347–357.
21. Barnes PJ. – Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 1995; 152: 838–852.
22. Llewellyn-Jones CG, Stockley RA. – The effects of β_2-agonists and methylxanthines on neutrophil function *in vitro*. *Eur Respir J* 1994; 73: 1460–1466.
23. Baldwin SR, Simon RH, Grum CM, Ketai LH, Boxer LH, Devall LJ. – Oxidant activity in expired breath of patients with adult respiratory distress syndrome. *Lancet* 1986; i: 11–14.
24. Kietzmann D, Kahl R, Müller M, Burchardi H, Kettler D. – Hydrogen peroxide in expired breath condensate of patients with acute failure and with ARDS. *Intensive Care Med* 1993; 19: 78–81.

25. Dohlman AW, Black HR, Royall JA. – Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. *Am Rev Respir Dis* 1993; 148: 955–960.
26. Linden M, Rassmussen JB, Pitulainen E, *et al.* – Airway inflammation in smokers with nonobstructive and obstructive chronic bronchitis. *Am Rev Respir Dis* 1993; 148: 1226–1232.
27. Williams MD, Chance B. – Spontaneous chemiluminescence of human breath. *J Biol Chem* 1983; 258: 3628–3631.
28. Cantin AM, Fells GA, Hubbard RC, Crystal RG. – Antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract. *J Clin Invest* 1990; 86: 962–971.
29. Habib MP, Clements NC, Garewal HS. – Cigarette smoking and ethane exhalation in humans. *Am J Respir Crit Care Med* 1995; 151: 1368–1372.
30. Green GM. – Mechanisms of tobacco smoke toxicity on pulmonary macrophage cells. *Eur J Respir Dis* 1985; 66 (Suppl. 139): 82–85.
31. Blue M, Janoff A. – Possible mechanisms of emphysema in cigarette smokers. Release of elastase from human polymorphonuclear leukocytes by cigarette smoke condensate *in vitro*. *Am Rev Respir Dis* 1978; 117: 317–325.
32. Greening AP, Downing I, Wood NE, Flenley DC. – Pulmonary antioxidants: catalase activity but not ceruloplasmin is increased in smokers. *Am Rev Respir Dis* 1985; 131: A385.
33. White CW, Ghezzi P, McMahon S, Dinarello CA, Repine JE. – Cytokines increase rat lung antioxidant enzymes during exposure to hyperoxia. *J Appl Physiol* 1989; 66: 1003–1007.
34. Visner GA, Dougall WC, Wilson JM, Burr IA, Nick HS. – Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor. *J Biol Chem* 1990; 265: 2856–2864.
35. Pietras T, Nowak D, Mazerant P, *et al.* – Effect of bacterial endotoxin on the activity of catalase and superoxide dismutase in selected organs of mice. *Current Pneumol* 1997; 1: 101–108.



Pavia, le Torri